academicJournals

Vol. 7(44), pp. 5082-5089, 7 November, 2013
DOI: 10.5897/AJMR2013.6003
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Isolation of salt tolerant endophytic and rhizospheric bacteria by natural selection and screening for promising plant growth-promoting rhizobacteria (PGPR) and growth vigour in tomato under sodic environment

T. Damodaran[1]*, V. Sah[2], R. B. Rai[2], D. K. Sharma[1], V.K.Mishra[1], S. K. Jha[1] and R. Kannan[1]

[1]Central Soil Salinity Research Institute, Regional Research station, Lucknow- 226005 India.
[2]Indian Veterinary Research Institute, Bareilly - 243122 India.

Accepted 8 October, 2013

The importance of plant growth promoting rhizobacteria in growth promotion and their ability to elicit 'induced systemic tolerance' against abiotic stresses has been documented. However, the performance of these microbes under various abiotic stresses especially saline-sodic conditions will be of great importance in the current agricultural scenario. In this study, we isolated 16 rhizobacteria through natural selection from saline sodic soils, and characterized them using morphological and biochemical parameters. These bacteria were assessed for their plant growth-promoting rhizobacteria (PGPR) traits like indole-3-acetic acid (IAA) production, ammonia and hydrogen cyanide (HCN) production, phosphate solubilization, etc. Furthermore, they were screened for *in-vitro* salt (NaCl) tolerance and Na^+ uptake pattern, where two stress tolerant rhizobacteria B-1 and B-3 identified as *Bacillus pumilus* and *Bacillus subtilis* showed all PGPR traits with tolerance to salinity. These isolates also elicited significantly higher vigor index in tomato seedlings grown in pot culture experiments under saline sodic soils of pH 9.35 and EC 4.2.

Key words: Rhizobacteria, salt tolerant, natural selection, PGPR.

INTRODUCTION

Rhizosphere is centre to microbial and nutrient dynamics and describes the zone of soil surrounding roots of plant species which release organic substances. Bacteria that are present in the rhizosphere and enhance plant growth by any mechanism are referred to as plant growth promoting rhizobacteria (PGPR) (Arnou et al., 1953). In both natural and man-made agro ecosystems, interactions between plants and soil micro-organisms have a profound effect on adaptation of plant to changing environment and plant growth (Kloepper et al., 1989; Bashan et al., 2004). Selections of microbial isolates from naturally stressed environment or rhizosphere are considered as possible measures for improving crop health which can control diseases and also promote plant growth (Lugtenberg and Kamilova, 2004; Mayak et al., 2004).

Worldwide, salinity is one of the most severe abiotic stresses that limit crop growth and productivity. Around 20% of worlds irrigated land is salt affected, with 2,500-5,000 km^2 of production lost every year as a result of

*Corresponding author. E-mail: damhort2002@yahoo.com.

salinity (UNEP, 2009). About 60% of salt affected soils are of sodic and saline sodic in nature which has increased steadily over decades in the northwest plains of the Indo-Gangetic basin and in China's Yellow River basin (Gupta and Abrol, 2000). High alkalinity (pH > 8.5) and high exchangeable sodium percentage (ESP>15) of the soil render it inhospitable for normal crop production and there is minimal bioproductivity in such soil (Chhabra, 1995). The utilization of salt-affected soil for agriculture has become necessary to meet the rise in food demand. One possible strategy to counteract the adverse effect of salinity is to exploit the avenues of bio-agents or bio-inoculants (Egamberdieva, 2012). Under salt stress, PGPR have shown positive effects in plants on parameters like germination rate, tolerance to drought, weight of shoots and roots, yield and plant growth (Raju et al., 1999). In an era of sustainable agricultural production, the interactions in the rhizosphere by soil micro-organisms with soil and plant plays a vital role in mobilization of nutrients from the limited pool (Mantelin and Touraine, 2004).

The combination of IAA production ability (Goldstein, 1995), phosphorous solubilization (Gyaneshwar et al., 1998) and siderophore production (Dulfy, 1994) of bacteria aid the plant rhizosphere in enhan-cing the nutrient absorption potential under sodic environ-ment for enabling economic production of commercial horticultural crops (Damodaran et al., 2013). This has been extensively attracting attention due to their efficacy as biological control and growth promoting ability in many crops. Though researchers earlier, have worked on isolation of salt (NaCl) tolerant rhizobacteria from halophytic environment where the conductivity (EC) of the soils is > 4 dsm^{-1}, little is known about their tolerance to saline sodic environment where the soils are severely affected by high pH characterized by high Na^+ in the soil solution phase as well as on cation exchange complex (Qadir and Schubert, 2002), exhibiting unique structural problems (slaking, swelling, and dispersion of clay). Therefore the present study is focused to survey plants habitat in the sodic soils and isolate rhizobacteria from sodic environment, to characterize and screen the isolates for PGPR and salt tolerant traits and further, to assess the growth vigor index of the tomato seeds primed with salt tolerant isolates in saline sodic soils.

MATERIALS AND METHODS

Survey of sodic soils

For the present experiment, a survey was conducted in 2009 in the Sharda Sahayak Canal Command areas of Rae Bareily district, Uttar Pradesh, India as it harbours major sodic belt. Collection of the rhizospheric soils and roots were made from halophylic plants grown in wild.

Physicochemical analysis of soil

The collected soil sample was analyzed for physicochemical parameters like pH and conductivity. The pH of the soil extract was determined potentio-metrically by an ORION ion analyzer (5 star series) using a pH electrode.

Isolation of rhizospheric and endophytic bacteria

The rhizospheric microbes were isolated from roots of phyto-ameliorant grasses collected from survey of undisturbed sodic site with prominent salt efflorescence as described by Quadt-Hallman et al. (1997). The soil samples were collected by fine brushing in sterile Petri dish for diluting and plating. Soils sample from rhizosphere of plants (10 g) were thoroughly mixed in 90 ml of autoclaved distilled water to make suspension. Soil suspension was kept for 30-60 min with periodic shaking. 1 ml of this suspension was added to 10 ml dilution vial and shaken. Serial dilution technique was performed up to 10^{-7} dilution. An aliquot of this suspension was spread on nutreint agar (NA) plate and incubated for 24-48 h at 28-30°C for observing colonies developed on it. Fine isolated colonies were picked up and streaked again on fresh NA plate and incubated similarly. This process was carried out thrice to get pure single colony.

Standard plate counting method

To enumerate the bacterial and fungal cultures, standard plate count method was used. The number of viable bacterial cells per unit volume of a sample using agar plate media was enumerated. The inoculum sample was spread across the plate and the colonies that were formed after incubation were counted. The colonies are referred to as colony forming units (CFU). Once the CFUs are counted on the plate, they were divided by the volume plated to determine the concentration of cells in the sample.

Bacterial identification

Bacteria were identified based on different morphological and staining characteristics. Based on the Gram staining property and cell morphology, the bacteria may be tentatively placed in four groups, that is, Gram +ve rods, Gram +ve cocci, Gram -ve cocci and Gram -ve rods. Usually, the predominant bacteria in rhizosphere of crop plants are Gram negative rods belonging to Gram -ve *Pseudomonas* and Gram +ve *Bacillus*. Further identification was done with specific biochemical tests (Cappunccino and Sherman, 1992).

In vitro analysis for the identification of PGPR strains

Production of indole acetic acid (IAA)

Indole acetic acid (IAA) production was detected as described by Brick et al. (1991). Bacterial cultures were grown for 72 h in nutrient broth media at 36- 38°C. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with 2 drops of orthophosphoric acid and 4 ml of Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). Development of pink colour indicates IAA production.

HCN production

Production of HCN was detected according to the method of Lorck (1948). Briefly, nutrient broth was amended with 4.4 g glycine / L and bacteria were streaked on modified agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate and 0.5% picric

Table 1. Enumeration of Rhizobacteria from soil samples by SPC method.

S/N	Location	Dilution	Amount of sample (ml)	Dilution factor (D)	Number of colony	Mean cfu per 100 mg soil	pH	Ece (dsm^{-1})
1	Kasrawa	10^{-3}	0.1	10^3	35	$45X10^3$	9.70	1.50
2	Hardoi	10^{-3}	0.1	10^3	5	$05X10^3$	10.2	4.55
3	Thakurenda	10^{-3}	0.1	10^3	45	$25X10^3$	10.0	1.24
4	Paschim Gau	10^{-3}	0.1	10^3	90	$90X10^3$	9.65	0.75
5	Gurbakshganj	10^{-3}	0.1	10^3	5	$35X10^3$	9.88	4.30

acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated at 36 ± 2°C for 4 days. Development of orange to red colour indicated HCN production.

Ammonia production

Bacterial isolates were screened for the production of ammonia in peptone water. Freshly grown culture were inoculated in 10 ml peptone water in each tube and incubated for 48-72 h at 28±2°C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive for ammonia production (Cappucino and Sherman, 1992).

Siderophore production

Bacterial culture (48 h) was streaked on nutrient agar medium amended with an indicator dye. The tertiary complex chrome-azurol-S(CAS) / Fe^{3+} / hexadecyl trimethyl ammonium bromide served as an indicator. Change of blue color of the medium surrounding the bacterial growth to fluorescent yellow indicated production of siderophore. The reaction of each bacterial strain was scored either positive or negative to the assay (Schwyn and Neilands, 1987).

Phosphate solubilization

Phosphate solubilization of isolates was evaluated from the ability to solubilize inorganic phosphate. Pikovskaya's agar medium containing calcium phosphate as the inorganic form of phosphate was used in assay. A loopful of bacterial culture were streaked on the plates and kept for incubation at 28°C for 4-5 days. The appearance of transparent halo zone around the bacterial colony indicated the phosphate solubilizing activity of the bacteria.

Salinity tolerance

For determining salt tolerance of the isolated bacteria, they were streaked on nutrient agar supplemented with 0.5, 5, 7.5 and 10% NaCl which acts as a selective medium. After the appearance of colonies, bacteria's were marked positive or negative for their ability to grow in different concentration of NaCl.

Sodium uptake

Potential isolates growing luxuriantly in 7.5% NaCl were screened for sodium uptake pattern. The isolates were grown overnight at 37°C in L-broth containing different NaCl concentration (0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 M). After 24 h, cells were harvested by centrifugation and bacterial pellet was washed with sterilized distilled water to remove the traces of medium. Washed pellet was digested overnight with 0.1 N HCl at room temperature. Samples were centrifuged and supernatant was taken for the estimation of uptake by bacterial cells. Sodium contents were measured by Flame photometer.

Determination of PGPR in sodic conditions

The rhizosphere and endophytic bacteria grown on nutrient broth with constant shaking on rotary shaker at 150 rpm for 48 h at room temperature (28±2°C) were harvested by centrifugation at 6000 rpm for 15 min. The bacterial cells were re-suspended in PB (0.01 M, pH 7.0). The concentration was adjusted using a spectrophotometer to approximately 10^8 CFU (OD_{595}=0.3) and used as inoculums for treating rice seeds (Thompson, 1996). Plant growth promoting activities of bacterial strains were assessed based on the seedling vigor index of tomato seed under pot culture studies in soil of pH 9.35, ECe of 4.2, Na^+ of 23.50 meq / l and sodium adsorption ratio (SAR) of 19.36. Sodium (Na^+) was determined by flame photometer (Richards, 1954) while sodium adsorption ratio (SAR) was determined by following generic equation:

$$SAR = \frac{Na}{\sqrt{(Ca + Mg)/2}}$$

The vigor index was calculated by using the formula as described by Abdul Baki and Anderson (1973):

Vigor index= Percent germination x Seedling length (shoot length + root length).

Statistical analysis

The pot culture experiment on assessing vigor index in tomato seeds treated with bacterial isolates was conducted in completely randomized design (CRD) with three replications and the data was analyzed using SAS 9.2 version. Prior to analysis of variance, the percentage values of germination were arcsine transformed.

RESULT

A transect survey was carried out in sodic lands of Rae Bareily district in Uttar Pradesh, India. The locations surveyed included Kasrawa, Hardoi, Thakurenkeda, Paschim Gau and Gurubakshganj (Table 1). These locations were observed to have extended patches of barren

Table 2. Morphological characteristics of isolated bacteria.

S/N	Experiment procedure	Observations And Result														
		G-1	G-2	-G-3	G-4	G-5	G-6	G-7	G-8	G-9	G-10	G-11	B-1	B-2	B-3	P-1	P-2
1	Colour	W	W	W	LW	Y	OW	LY	C	W	W	Y	CW	CW	C	Y	LY
2	Shape	I	S	Co	S	R	R	S	R	I	R	R	R	R	R	R	R
3	Elevation	Cv	Ra	Cv	Ra	Ra	Ra	Ra	Ra	F	Ra	Ra	Ra	Ra	Ra	Ra	Ra
4	Pigmentation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	B	B

W=White, LW= light white, Y= yellow, O=off white, LY= light yellow, C=creamy off white, CW= creamy white, I= irregular, S= spherical, Co= cocci, R= round, Ro= rod, Cv= convex, Ra= raised, F= flat, B= bright, N= none.

sodic soils with pH ranging from 9.65 (Paschim Gau) to 10.2 (Hardoi). The population (cfu gm^{-1}) of bacteria ranged from 90 $\times 10^3$ (Paschim Gau) to 5 $\times 10^3$ (Hardoi). About 32 bacterial isolates were collected and among them 16 were selected and sorted out into pure different colonies, exhibiting morphological and staining characteristics (Tables 2 and 3). Among the 16 isolates, five (G-5, G-7, G-11, P-1 and P-2) were yellowish to light yellowish colour while the others were white to creamy white in colour. The isolates were of irregular, spherical, cocci, round and rod shaped. Pigmentation was absent in most of the isolates except P-1 and P-2 which displayed bright pigments.

Further, they were screened for Gram nature, motility, pigmentation, colony and morphological characteristic. Among the 16 isolates, six were gram positive and 10 were Gram negative (Table 3). Results have indicated that 10 out of 16 isolates were able to assimilate starch, glucose and fructose, while seven were able to assimilate nitrate and 8 were positive for indole production.

PGPR traits

Sixteen isolates were further screened for PGPR traits (Table 4) like IAA, siderophore, ammonia and HCN production and also phosphate mobilization ability. Among them, seven showed IAA and HCN production. The isolates B-1 and B-3 had extensive zone formation for IAA (>1cm). Four isolates showed siderophore production with two of them (B-1 and B-3) belonging to genus *Bacillus* having much higher zone ranging from 0.6 - 0.9 cm.

Production of ammonia was detected in nine isolates and phosphate solubilization zone was observed in eight isolates. Four isolates B-1, B-3, P-1 and P-2 showed higher phosphate soulubilization (0.6- 0.9 cm). Among the 16 isolates screened, two (B-1 and B-3) of them exhibited positive response to all the *in-vitro* PGPR characteristics studied.

Salt tolerance traits

On screening all the 16 bacterial isolates for growth in different NaCl concentrations; five isolates (B-1, B-2, B-3, P-1 and P-2) growing luxuriantly in 7.5 % NaCl concentration were selected for further evaluations (Table 5). These five isolates also exhibited halo formation when grown on Mannitol salt agar medium containing 7.5% NaCl. Furthermore, analysis of these isolates for sodium uptake pattern (Figure 1) at different molar (M) concentration of NaCl showed an increasing sodium (Na^+) uptake up to 1 M NaCl in all the isolates beyond which there was a significant decline. However, among them, two isolates B-3 and B-1 identified as *Bacillus pumilus* and *Bacillus subtilis* showed higher uptake of Na^+ (1.272 meq / L and 1.122 meq / L respectively) at 1 M NaCl concentration.

Screening of the salt tolerant isolates for plant growth potential (PGP) in saline-sodic soils of pH 9.35 and EC 4.2 dsm^{-1} under pot culture experiment of tomato var. Himsona showed that among the five, B-1 and B-3 had plant growth enhancing activities with the germination percentage of 95.0 and 93.0% (Table 6). Significantly higher shoot and root growth were observed in B-3 followed by B-1 with higher vigour index (1900.0 and 1525.2 respectively).

DISCUSSION

A detailed survey of the natural population in the rhizosphere of grasses grown in sodic soils was carried out in the present study to isolate and identify salt tolerant bacteria that could express plant growth promotion (PGP) traits at high salt concentrations. Our studies establish that the bacterial diversity reduce with increase in soil pH under natural selection sites. It has been reported

Table 3. Identification of potential bacterial isolates based on bio-chemical tests.

S/N	Parameter	G-1	G-2	G-3	G-4	G-5	G-6	G-7	G-8	G-9	G-10	G-11	B-1	B-2	B-3	P-1	P-2
1	Gram staining	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Gelatin liquefaction	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	+
3	Catalase test	-	+	_	+	+	-	-	+	+		-	+	+	+	+	+
4	Oxidase test	-	_	_	-	-	-	+	-	-		+	-	-	-	+	+
5	Starch hydrolysis	-	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-
6	Fluorescent pigment	-	_		-	-	-	-	+	-	-	-	-	-	-	+	+
7	Indole	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+	+
8	Methyl red	+	-	_	-	-	-	+	+	-	+	-	-	-	-	+	+
9	VP	+	+	+	+	+	+	-	-	+	-		+	+	+	-	-
10	Citrate	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-
11	Nitrate	_	_	_	+	+	-	-	-	+	+	-	+	+	+	-	-
12	Glucose	A^+	A^-	A^+	A^+	A^+	A^+	A^+	A^+	A^-	A^-	A^-	A^+	A^+	A^+	A^-	A^-
13	Fructose	A^-	A^+	A^+	A^+	A^+	A^+	A^+	A^+	AP	A^-	A^-	A^+	A^+	A^+	A^-	A^-
Identification of the isolates		A	B	C	D	E	F	G	H	I	J	K	M	L	N	O	P

A = *Serratia* sp.; B = *Micrococcus* sp.; C = *Azotobacter* sp.; D = *Bacillus safensis;* E = *Bacillus subtilis;* F = *Rhizobium;* G = *Bacillus* sp.; H = *Pseudomonas* sp.; I = *Brevibacillus* sp.; J = *Azospirillum* sp.; K = *Uncultured bacterium;* L = *Bacillus pumilus*; M = *Bacillus cereus*; N = *Bacillus subtilis*; O = *Pseudomonas putida*; P = *Psuedomonas* sp.; A^+ = Acid producers; A^- = No acid producers.

Table 4. Assessment of plant growth promoting bacteria for different growth promotion traits.

S/N	Experiment procedure	Observations and result															
		G-1	G-2	-G-3	G-4	G-5	G-6	G-7	G-8	G-9	G-10	G-11	B-1	B-2	B-3	P-1	P-2
1	IAA production	+	-	-	-	-	-	-	+	-	+	-	+++	-	+++	++	++
2	HCN	+	-	-	-	-	-	-		+	+	-	+	+	+	-	+
3	Siderophore	_	+	-	-	-	-	-	-	-	-	-	++	-	++	-	+
4	Ammonia	+	+	+	+	+	-	-	-	-	+	-	+	-	+	+	-
5	P- solubilization	+	-	-	-	-	+	+	-	-	+	-	++	-	++	++	++

IAA, Indole-3-acetic acid; HCN, hydrogen cyanide; -, no production; +, 0.3-0.5 cm; ++, 0.6-0.9 cm; +++, >1 cm.

earlier that soil salinity plays a prominent role in the microbial selection process as environmental stress leads to reduce bacterial diversity (Borneman et al., 1996). In our study, we have isolated 32 isolates from natural selection in the rhizopshere of grasses grown in sodic soils and sorted them into 16 different pure colonies. Majority of the bacterial isolates are identified as *Bacillus* spp. based on biochemical and morpholo-gical observations. Earlier studies show that genera such as *Bacillus* and *Pseudomonas* tend to be pre-dominant in saline soils (Tank and

Table 5. Screening of isolates for tolerance to salinity.

S/N	NaCl concentration	Observations and result (48 h)															
		G-1	G-2	-G-3	G-4	G-5	G-6	G-7	G-8	G-9	G-10	G-11	B-1	B-2	B-3	P-1	P-2
1	Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	0.5%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	5%	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+
4	7.5%	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
5	10%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = Luxiriant, - = no growth

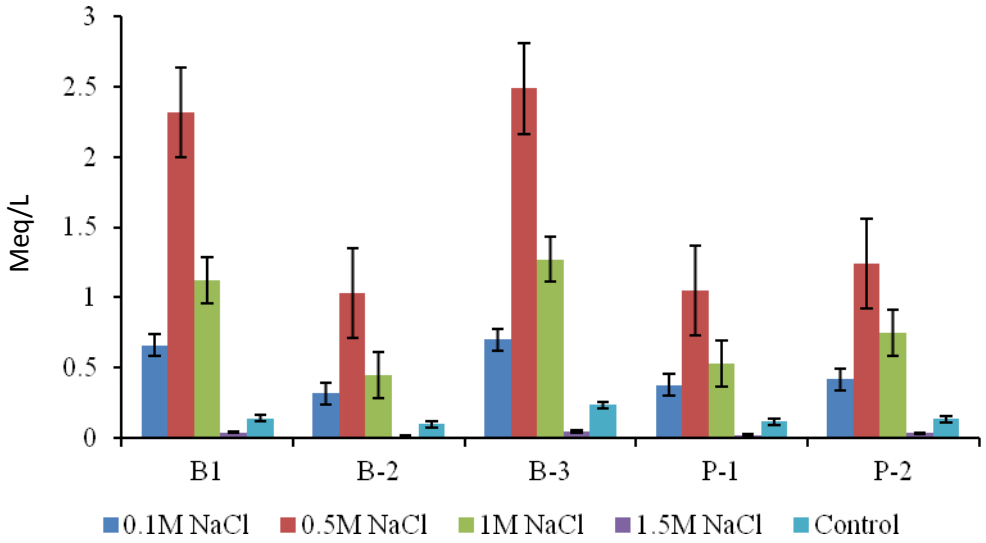

Figure 1. Sodium uptake pattern of the elite PGPR strains of sodic soils. Vertical bars indicate ±standard error

Saraf, 2010).

PGP activity of the bacteria present in the rhizosphere is found to exert beneficial effects on plant growth mechanism. Several mechanisms such as production of phytohormones, suppression of deleterious organisms, production of IAA, activation of phosphate solubilization and promotion of the mineral nutrient uptake are believed to be involved in plant growth promotion by PGPR (Glick, 1995). IAA, the most common auxin function as important signal molecule in the regulation of plant development (Usha Rani et al., 2012). Out of 16 isolates in our present study seven exhibited IAA production which can attribute significant growth enhancement in plants.

Phosphorous (P) is an essential nutrient for plant growth, development and is typically insoluble or poorly soluble in soils under salt stressed conditions (Harrison et al., 2002). Some of the bacteria are known to improve the solubilization of the fixed soil phosphorous and applied phosphates, resulting in higher yields even under stress conditions (Banerjee et al., 2010). In our experiment, eight rhizobacterial isolates showed *in-vitro* phosphate solubilizing efficiency and has been tested in plant growth. Ability to solubilize various insoluble phosphates is always desirable attribute for a competent PGPR. Phosphate solubilization by *Bacillus sp.* isolated from salt stressed environment had been observed by earlier researchers (Son et al., 2006).

Siderophore chelates iron and other metals contributing to disease suppression and acquisition of Fe^{2+} to plants for increasing the crop growth under stressed conditions (Hofte et al., 1992; Duffy, 1994). Our study shows four isolates with siderophore production ability which will be a productive PGPR trait for selection. Production of ammonia (Wani et al., 2007) and HCN (Schippers et al., 1990) is an important attribute of PGPR that influences plant growth indirectly and strengthen the host disease resistance mechanism respectively. In our present study, nine isolates produce ammonia and seven produce HCN. Majority of the ammonia producing bacteria were identified to be of genus *Bacillus* and *Pseudomonas* spp. Production of ammonia was commonly detected in the isolates of *Bacillus* and *Pseudomonas* (Joseph et al., 2007).

Out of the 16 bacteria isolated from sodic rhizospheres, five showed tolerance to high salt concentration (7.5 % NaCl) and among them isolate B-1 and B-3 had higher uptake of sodium when cultured under *in-vitro* conditions in 1 M NaCl solution. It has also been reported previously that bacteria isolated from saline soil are more likely to withstand saline conditions (Upadhyay et al., 2009). On the other hand, if such bacteria also possess plant growth promoting traits, they would be ideal for use in sustainable agriculture (Egamberdiyeva and Islam, 2008). Therefore, the two salt tolerant bacterial isolates B-1 and B-3 identified as *B. pumilus* and *B. subtilis* also exhibited positive response for *PGPR* characteristics like IAA, HCN, siderophore, HCN and ACC deaminase production. Production of IAA, siderophore, phosphate solubilization had been observed in *Bacillus* and *Pseudomonas sp*. in earlier studies (Xie et al., 1996; Loper and Henkels, 1997). Furthermore, in the current experiment, the assessment of vigor index of tomato seeds treated with five salt tolerant isolates (B-1, b-2, B-3, P-1 and P-2) showed that the isolates B-1 and B-3 are potential growth promoter with higher vigour index even under saline-sodic conditions apart from salinity. Though PGPR are more commonly known to induce resistance against pathogen infection, reports are now available on their ability to elicit 'induced systemic tolerance' against abiotic stresses (Usha et al., 2011).

In this study, we have shown that two strains B-3 and B-1 identified as *B. pumilus* and *B. subtilis* isolated from the rhizosphere of grasses in sodic soils of high pH are efficient for the tested salt tolerant traits under saline and sodic conditions. They also showed positive response for PGPR traits like IAA production, phosphate solubilizaiton, etc. These two isolates show potential as plant growth beneficial inoculants in alkaline soil regions suggesting further studies on rhizocompetence in commercial crops grown under salt stressed conditions.

REFERENCES

Abdul Baki AA, Anderson JD (1973). Vigour determination in soybean seed by multiple criteria. Crop Sci. 13:630-633.

Arnou DI (1953). Soil and fertilizer phosphorus in crop nutrition (IV). In: (Eds.) Pierre WH and Noramn AG. Acad. Press NY.

Banerjee G, Scott-Craig JS, Walton JD (2010). Improving enzymes for biomass conversion: a basic research perspective. Bioenerg. Res. 3:82-92.

Bashan Y, Holguin G, de-Bashan L (2004). *Azospirillum*-plant relationships: physiological, molecular, agricultural and environmental advances. Can J. Microbiol. 50:521-577.

Borneman J, Skroch PW , O'Sullivan KM , Paulus JA , Rumjanek NG, Jansen JL, Nienhuis J , Triplett EW (1996). Molecular microbial diversity of an agricultural soil in Wisconsin. Appl. Environ. Microbiol. 62: 1935 - 1943.

Brick JM, Bostock RM, Silverstone SE (1991). Rapid *insitu* assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. Appl. Environ. Microbiol. 57:535-538.

Cappuccino JC, Sherman N (1992). In: Microbiology: A Laboratory Manual 3rd ed New York: Benjamin/Cumming Pub.Co.

Chhabra R (1995). Nutrient requirement for sodic soil. Fertil. News 40:13-21.

Damodaran T, Mishra VK, Sharma, DK, Jha SK, Verma CL, Rai RB, Kannan R, Nayak AK and Dhama K (2013) Management of sub-soil sodicity for sustainable banana production in sodic soil - an approach. Int. J. Curr. Res. 5 (7):1930-1934.

Duffy BK (1994). Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. Appl. Environ. Microbiol. 2429-2438.

Egamberdieva D (2012). *Pseudomonas chlororaphis*: a salt-tolerant bacterial inoculant for plant growth stimulation under saline soil conditions. Acta Physiol. Plantarum 34:751-756.

Egamberdiyeva D, Islam KR (2008). Salt-tolerant rhizobacteria: plant growth promoting traits and physiological characterization within ecologically stressed environments. In: Ahmad I, Pichtel J, Hayat S (eds) Plant-bacteria interactions- strategies and techniques to promote plant growth. Wiley 257-281

Glick BR (1995). The enhancement of plant growth by free living bacteria. Can. J. Microbiol. 41:109-114.

Goldstein AH (1995). Evidence for mutualism between a plant growing in a phosphate-limied desert environment and a mineral phosphate solubilizing rhizobacteria. pp. 295-300.

Gupta RK, Abrol IP (2000). Salinity build-up and changes in the rice-wheat system of the Indo-Gangetic Plains. Exp. Agr. 36:273-284.

Gyaneshwar P, Naresh KG, Parekh LJ (1998). Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245:83-93.

Harrison MJ, Dewbre GR, Liu J (2002). A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. Plant Cell 14:2413-2429.

Hofte M, Boelens J, Verstraete W (1992). Survival and root colonization of mutants of plant growth promoting *Pseudomonads* affected in siderophore biosynthesis or regulation of siderophore production. J. Plant Nutr. 15:2253-2262.

Joseph B, Patra RR, Lawrence R (2007). Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer*

ariethium L.). Int J.Plant. Prod. 2:141-152.
Kloepper JW, Lifshitz R, Zablotowicz RM (1989). Free-living bacterial inocula for enhancing crop productivity. Trends. Biotechnol. 7:39-43.
Loper JE, Henkels MD (1997). Availability of iron to *Pseudomonas fluorescence* in rhizosphere and bulk soil evaluated with an ice nucleation reporter gene. Appl. Environ. Microbiol. 63:99-105.
Lorck H (1948). Production of hydrocyanic acid by bacteria. Plant. Physiol. 1:142 -146.
Lugtenberg BJJ, Kamilova FD (2004). Rhizosphere management: microbial manipulation for biocontrol. In Goodman RM (eds) Encyclopedia of plant and crop science. Marcel Dekker, New York. pp.1098-1101.
Mantelin S, Touraine B (2004). Plant growth-promoting bacteria and nitrate availability impacts on root development and nitrate uptake. J. Exp. Bot. 55:27-34.
Mayak S, Tirosh T, Glick BR (2004) .Plant growth-promoting bacteria that confer resistance in tomato and pepper plants to salt stress. Plant Physiol. Biochem. 167:650-656
Qadir M, Schubert S (2002). Degradation processes and nutrient constraints in sodic soils. Land Degradation Development. 13:275-294.
Quadt-Hallman A, Hallman J, Mahafee WF, Kloepper JW (1997). Bacterial endophytes in agricultural crops. Can. J. Microbiol. 43:895-914
Raju NS, Niranjan SR, Janardhan GR, Prakash HS, Mathur SB (1999). Improvement of seed quality and field emergence of *Fusarium moniliformae* infected sorghum seeds biocontrol agents. pp. 206-212.
Richards LA (1954). Diagnosis and improvement of saline and alkali soils. US Salinity Lab., US Department of Agriculture Handbook 60. California, USA.
Schippers B, Bakker AW, Bakker R, Van Peer R (1990). Beneficial and deleterious effects of HCN-producing pseudomonads on rhizosphere interactions. Plant Soil 129:75- 83.
Schwyn B, Neilands JB (1987).Universal chemical assay for detection and determination of siderophore. Ant. Biochem. 160:47-56
Son HJ, Park GT, Cha MS, Heo MS (2006). Solubilization of insoluble inorganic phosphates by a novel salt- and pH tolerant *Pantoea agglomerans R-42* isolated from soybean rhizosphere. Bioresour. Technol. 97:204-210.
Tank N, Saraf M (2010). Salinity resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. J. Plant. Interactions. 5 (1):51-58.
Thompson DC (1996). Evaluation of bacterial antagonist for reduction of summer patch symptoms in Kentucky blue grass. Plant Disease. 80:856-862.
UNEP (2009).The environmental food crisis: The environments' role in averting future food crisis.
Upadhyay SK, Singh DP, Saikia R (2009). Genetic Diversity of Plant Growth Promoting Rhizobacteria Isolated from Rhizospheric Soil of Wheat Under Saline Condition. Curr. Microbiol. 59:489 496.
Usha RM, Arundhathi, Reddy G (2011). *Bacillus cereus* and *Enterobacter cancerogenus* screened for their efficient plant growth promoting traits rhizobacteria (PGPR) and antagonistic traits among sixteen bacterial isolates from rhizospheric soils of pigeon pea. A. J. Microbial. R. 5(15):2090-2094
Usha RM, Arundhathi, Reddy G (2012). Screening of rhizobacteria containing plant growth promoting (PGPR) traits in rhizosphere soils and their role in enhancing growth of pigeon pea. A. J. Biotechnol. 11(32):8085-8091.
Wani PA, Khan MS, Zaidi A (2007). Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp (vigna) on growth, symbiosis, seed yield and metal uptake by green gram plants. Chemosphere 70:36-45.
Xie H, Pasternak JJ, Glick BR (1996). Isolation and characterization of mutants of plant growth promoting rhizobacterium *Pseudomonas putida* GR 12-2 that over produce indoleacetic acid. Curr. Microbiol. 32:67-71.